More broadly, however, we need to get the message to the ordinary taxpayer and to legislators of the importance of basic research and of stable, long-term funding for research that only governments can afford to support. I wonder if we could convince Fox News in the USA to have a 'redneck scientist' segment in which a 'salt of the earth' scientist explains to the Fox audience how basic research helps *them* individually.

You mentioned that music led you to science. Do you see any parallels between these two spheres? Music continues to be important to me. I sing with the Nashville Symphony Chorus, and the rehearsals and performances of the classical choral repertoire are a regular series of oases in my life. On the one hand, I am just 1 out of 145 voices and am never recognized individually. On the other hand, it is a privilege and pleasure for me to have the opportunity to be a part of a program with professional musicians (they are totally out of *my* league). My choral participation is a metaphor for the way I view my ultimate contribution to science. There are few scientists who achieve name recognition status, and almost all of those who do reach that stature during their research-active years are practically forgotten within a few years after their retirement. For example, I have been shocked that the work and contributions of my graduate supervisor (Pittendrigh) — a dominant figure of chronobiology who died 17 years ago — are rarely discussed nowadays. Therefore, lasting personal recognition is not a realistic motivation for becoming a researcher/teacher. However, we contribute to a process that is larger than ourselves. In that sense, we are each a 'voice in the chorus'. While I was initially attracted to science by the fallacy that I might be recognized as having accomplished great things, now my goals are more realistic: firstly, in my teaching, I can influence the lives and decisions of hundreds of students every year in ways that will be largely unknowable to me and, secondly, in my research, I can enjoy an endeavor that remains fresh and challenging as new experimental results force us to continuously re-evaluate previous conclusions.

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Quick guide

Precision genome engineering

Dana Carroll

What is precision genome engineering? Exactly that! You know what a genome is; 'engineering' means that we want to change that DNA, and 'precision' means we want to do it in a very specific, targeted way.

Sounds challenging, why would you want to do that? Lots of reasons. For example, we might want to make a mutation in a gene in an experimental organism in order to see what the effect was and thus get an insight into the gene's function. With real precision, we could introduce an exact mutation that corresponds to a human genetic disease allele into an experimental organism. That would allow us to examine the physiological consequences of that mutation in more detail than we could just by looking at people.

Are there some more practical, real-world applications? You bet! People are using precision genome engineering with crop plants and food animals to give them improved characteristics. Think about maize that was more drought resistant, or canola that produced more beneficial oil. How about pigs with more muscle mass — call that pork — or dairy cows with no horns to bother their sisters or the farmer. These modifications are already under way.

How can you get these changes to be made efficiently and with real specificity? The key is to use proteins that will cut DNA — called nucleases — and to direct them to exactly the place in the genome that you want to modify. The first engineered proteins of this kind were called zinc-finger nucleases, or ZFNs. They have a nuclease attached to zinc finger modules that come from natural DNA binding proteins (mainly transcription factors) and know how to find and bind to very specific DNA sequences. There are natural and synthetic fingers that in the right combination can recognize quite a range of DNA sequences. About four years ago, another type of DNA-binding module was characterized that has a very simple way of recognizing DNA — one module for each base pair. The nucleases made from these are called TALENs (transcription activator-like effector nucleases), and they have stolen quite a bit of territory from ZFNs.

So these proteins make a cut in the DNA strand, but what happens then? Once a break is made at a specific site by the nuclease, the cell's double strand repair machinery hurries to fix it and sometimes makes a mistake. This introduces a mutation right at the break site, and often knocks out the function of a gene. Another type of repair uses a DNA template to copy information across the break. If we put into cells a template that carries sequence changes we want to introduce, they will often get copied in. That's how you would put in a human disease mutation, for example. Together ZFNs and TALENs have been used successfully to modify the genomes of about 30 different species, including humans.

Humans? You're messing with my genome? No (not yet, but stay vigilant). Lots of genome engineering has been done in cultured human cells, partly to make disease models, partly just to work out the technology. Ultimately we want to use these nucleases for beneficial gene therapy. Right now there are clinical trials going on with ZFNs targeted to the human CCR5 gene. The product of this gene is a protein that the human immunodeficiency virus (HIV) needs in order to infect T cells. It turns out we can get along without this protein — there are natural CCR5 mutants. The therapy is to take T cells from HIV-infected people, treat them in the lab to knock out CCR5, then put them back into the same person. This will prevent the development of AIDS by providing a population of HIV-resistant T cells, and there won't be a rejection problem because the cells came from the same person who receives them.

Sounds amazing, but what can you do besides help AIDS patients? The things that look easiest right now are ones where the nuclease treatment

can be done outside the body — *ex vivo*, as we say. Any condition involving blood cells would be a candidate: thalassemia (hemoglobin deficiencies), severe combined immune deficiencies (SCID), and others. When it comes to delivering the nucleases to intact organs, the challenge becomes greater, but people are working on it. Researchers are also using these nucleases to modify human stem cells in culture. As we learn enough about such cells to have some confidence in putting them into the body, we will also have the tools to correct genetic defects in them. You can imagine manipulating neural stem cells in culture to help reverse neurodegenerative diseases that have a genetic cause.

What's next? There is a lot of work ahead to bring the applications I've hinted at to fruition. But beyond the slogging, there is a new nuclease technology, with the catchy name of CRISPR. This system uses a single bacterial protein to do the DNA cutting, and it is guided to its target by an RNA molecule using Watson-Crick base pairing. In that way, it is simpler than ZFNs and TALENs, and it threatens to take over entirely. The CRISPR approach has already been used in a number of systems very effectively, but there are some concerns about whether it will be specific enough for use in people. Keep your eyes open. The future will also be full of surprises.

Where can I find out more?

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Primer

Rhizaria

Fabien Burki and Patrick J. Keeling

Have you ever stumbled across Ernst Haeckel's stunning 19th century art prints representing complex symmetrical forms that look like snowflakes, armored knights, or even futuristic space stations? Or maybe walking down an indo-pacific beach, you have taken a closer look at the warm sand only to realize that the 'sand' is really countless, minute earthly stars? Chances are you did not realize it, but in both cases you were looking at the skeletons of single-celled organisms belonging to Rhizaria, a large group, or 'supergroup', of eukaryotes. Various kinds of rhizarians have long been known to biologists, as evidenced by the fame and frequency with which Haeckel's illustrations have been reproduced, but the idea that these organisms are all related to one another emerged only recently. And this means that Rhizaria, as a whole, is one of the most poorly understood supergroups of eukaryotes.

That Rhizaria lags far behind more studied groups is of course a challenging statement to prove, but a fair approximation to illustrate the depth of our knowledge, or rather the lack of it, is to look at the available genetic and genomic information. Figure 1 depicts our current understanding of the evolutionary tree of eukaryotes, as assembled by more than 20 years of molecular phylogenetics and advanced microscopy. In addition to positioning Rhizaria among its 'peers', this figure also shows how researchers have allocated the genomic resources across the whole phylogenetic spectrum of eukaryotes. In this tree, the thickness of each branch corresponds to the number of complete nuclear genomes available from members of those lineages collectively. Looking at the last common ancestor of eukaryotes (here at the center, or the unresolved base of the tree), one can see that we have sequenced a lot of nuclear genomes, but as we move out to the tips it becomes apparent that most sequencing effort has focused on a few lineages only, primarily animals, fungi, plants, and their respective parasites. Microbial eukaryotic lineages (aka the protists) are generally undersampled, but with only one complete genome available, that of the chlorarachniophyte *Bigelowiella natans* and the foraminifer *Reticulomyxa filosa*, the entire supergroup Rhizaria still stands out among protists in the extreme paucity of genomic data. It is not surprising that large, multicellular organisms are better studied, as these have always been the main subjects of biological research. By extension, parasites of animals and plants have also attracted much attention, and among protists the vast majority of sequenced genomes are from pathogens of humans or economically important animal and plant species. For example, the apicomplexan parasites, such as the malaria agent *Plasmodium falciparum*, top the list of protist groups with complete genomes (currently about 30). Another, slightly weaker bias comes from our preference for sequencing genomes from photosynthetic species, for instance green and red algae with 11 and 5 genomes, respectively, or diatoms with 4 genomes (Figure 1).

So why is Rhizaria so understudied? One reason is that they almost entirely run contrary to the above noted biases. For a start, only two relatively small subgroups are photosynthetic, one being the chlorarachniophytes, which not surprisingly include one of the two rhizarian genomes currently available (*B. natans*). Moreover, no rhizarian parasite of human is currently known, and only a handful parasitize commercially relevant species, mostly crops or invertebrates (see below). Aside from these biases, Rhizaria also faces a problem of a more technical nature: they are hard to cultivate, and until recently a relatively large scale cell culture was a prerequisite for launching major sequencing projects in order to meet the material (DNA or RNA) requirements.

So does that mean that rhizarian genomics, and by extension rhizarian research as a whole, faces a dark future, being labeled 'difficult and not sufficiently sexy'? On the contrary, our awareness of the global significance of Rhizaria is on the rise, just as the technical difficulties are rapidly receding. Rhizaria itself represents a fascinating reservoir of unexplored diversity. At the environmental and